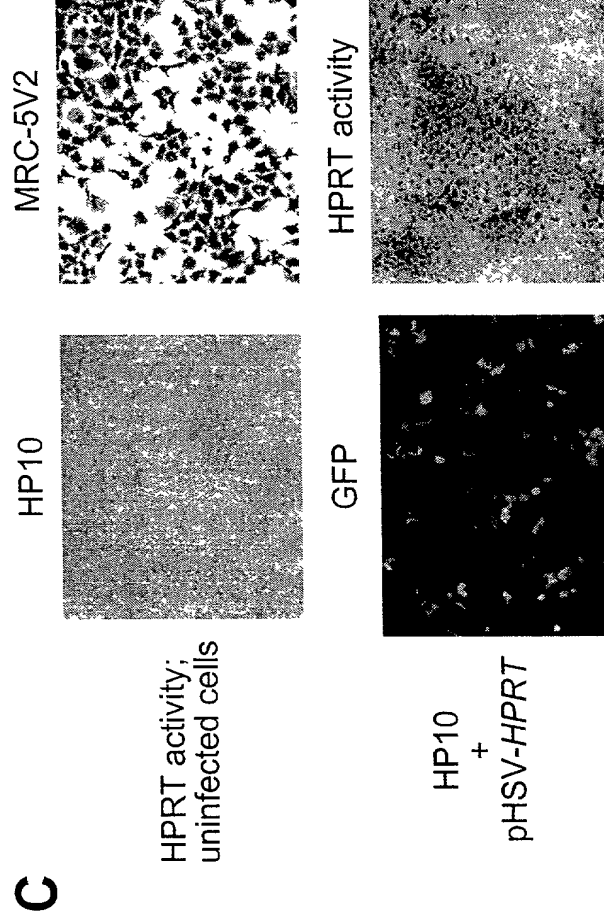
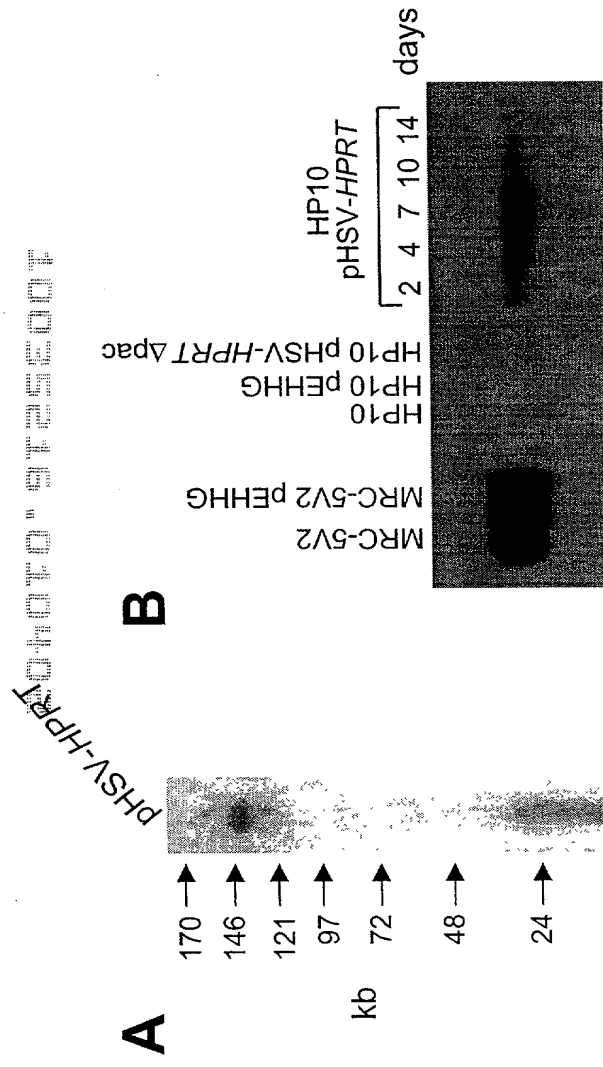
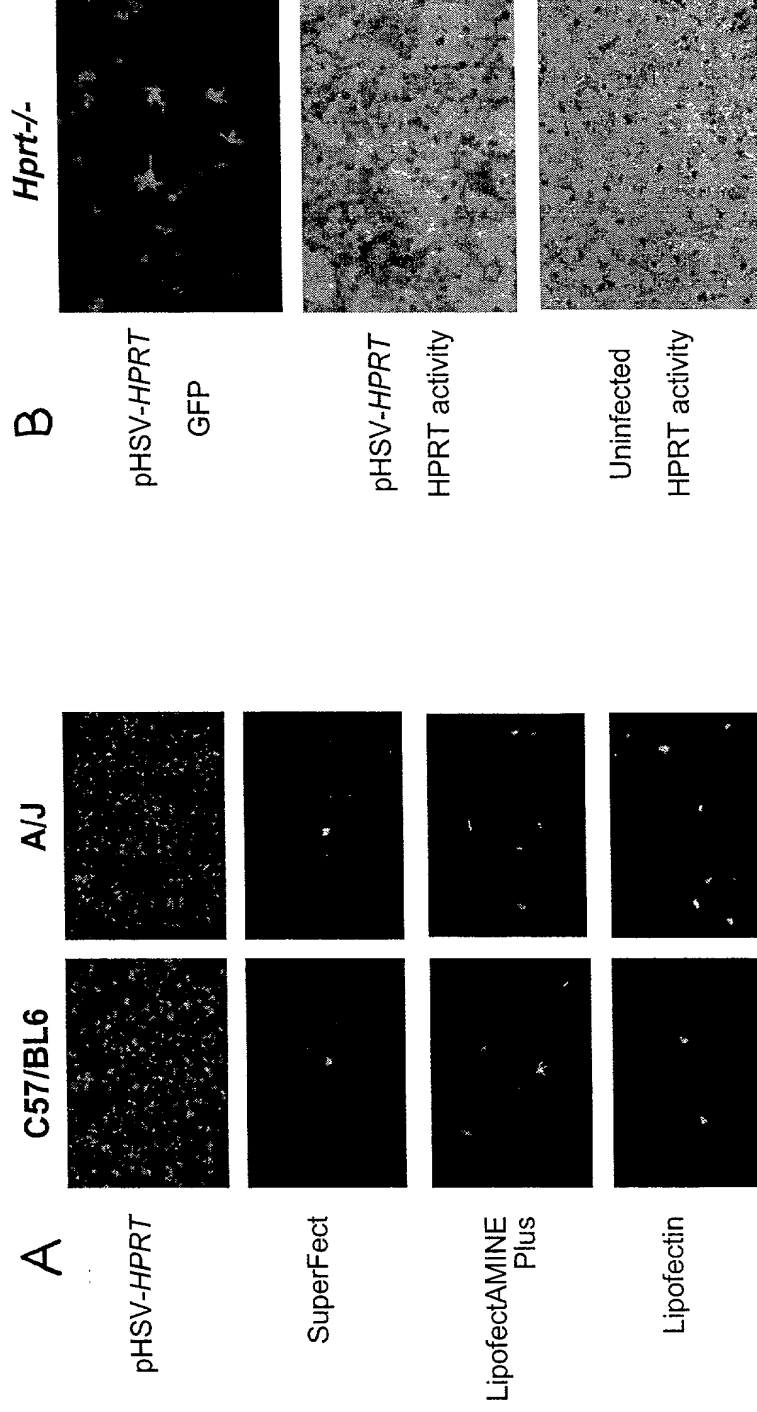


Figure 1



**Figure 2**



**Figure 3**

## Figure 4



# Construction and infectious delivery of *LDLR* BAC genomic DNA vectors

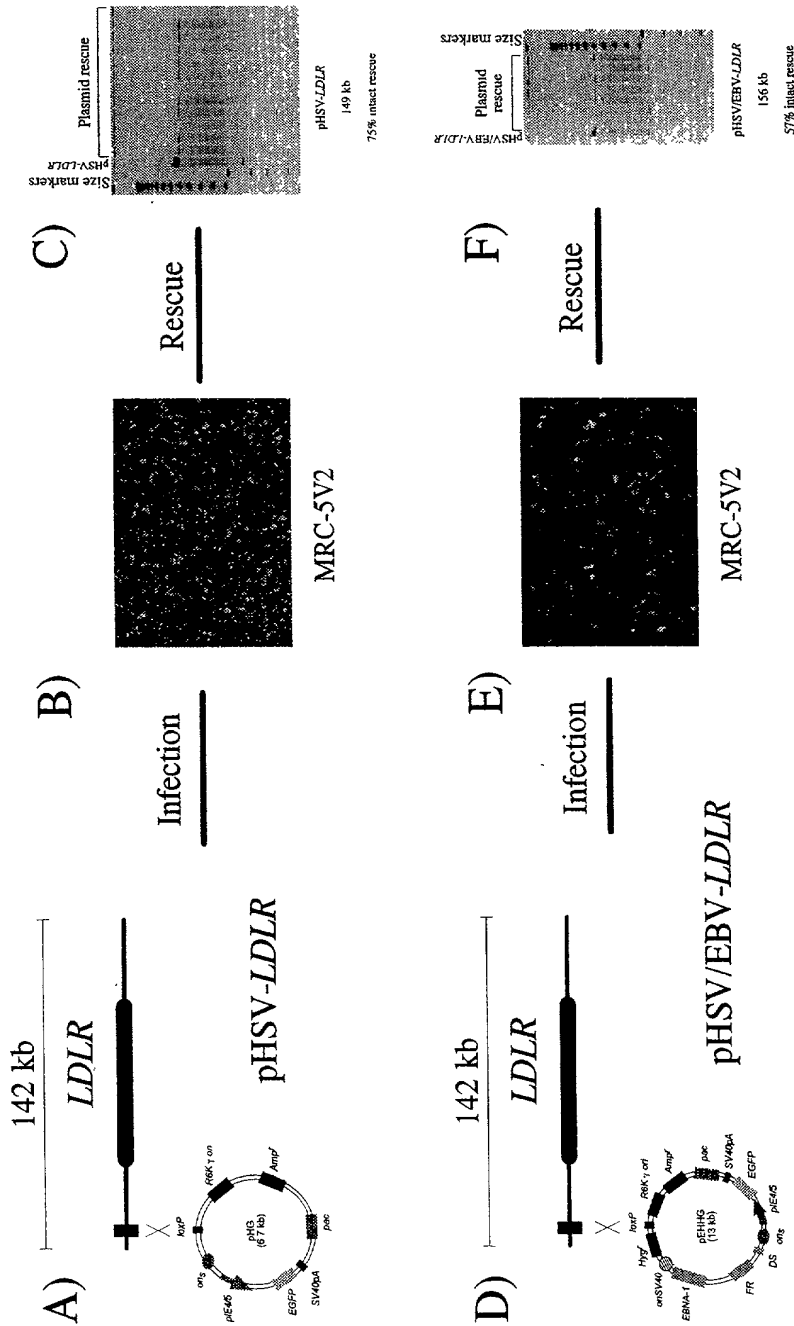
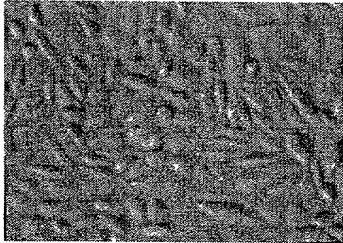


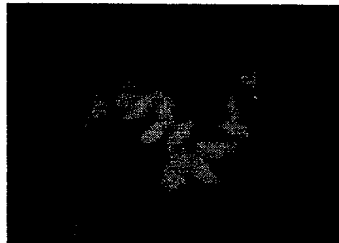
Figure 5

**Functional infectious delivery of the  
human *LDLR* genomic DNA locus**

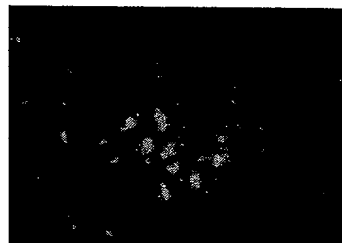
A) Light



B) GFP



C) LDLR



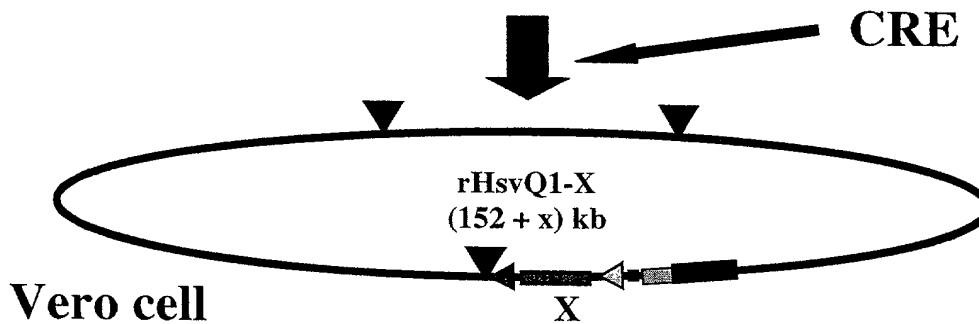
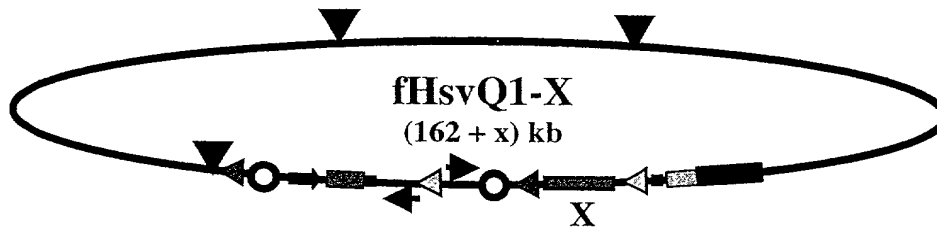
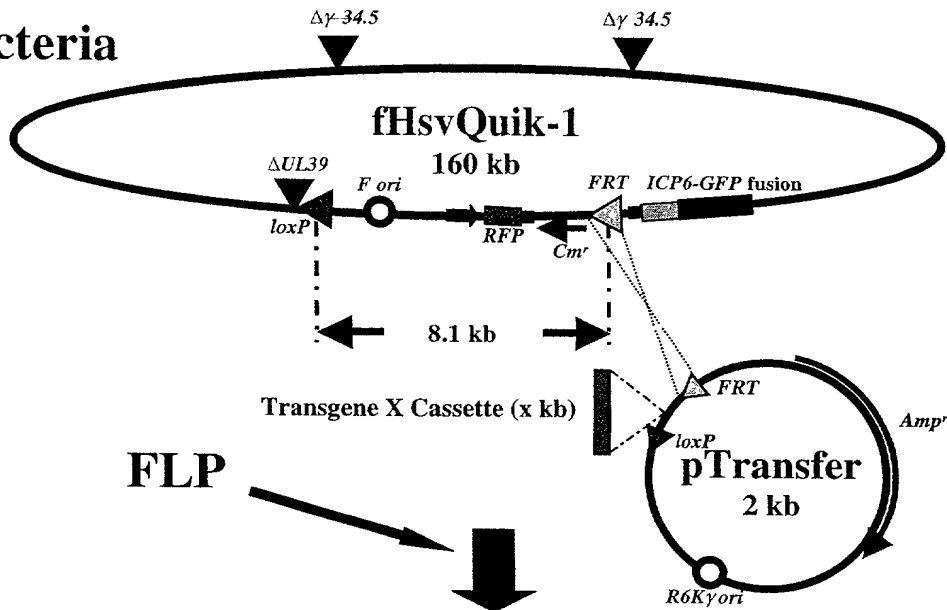
*ldlr*<sup>-/-</sup> a7 CHO cells + pHSV-*LDLR* (149 kb)

Figure 6

2010-09-15 10:00

# HsvQuik System

**Bacteria**



**rHsvQ1-X**

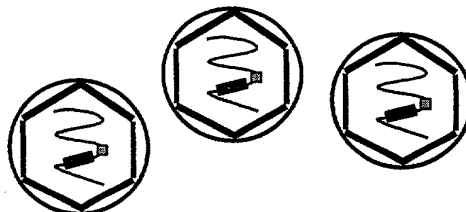
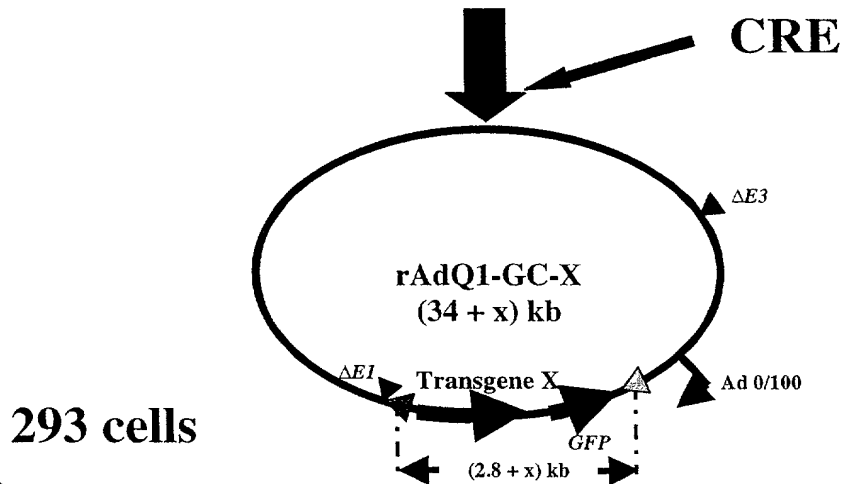
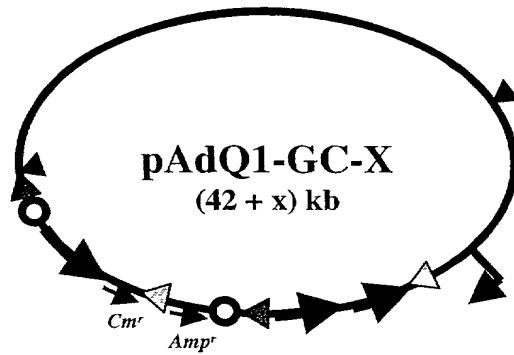
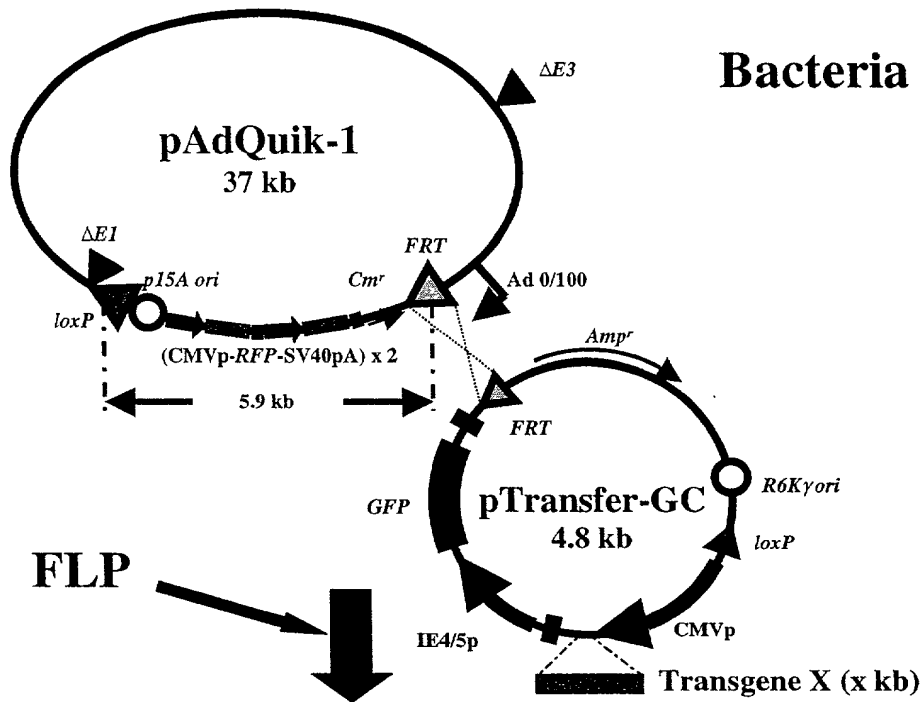


FIGURE 7

# AdQuik System



**rAdQ1-GC-X**

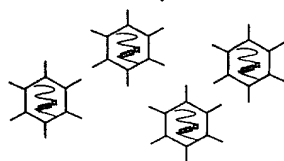


FIGURE 8



# Restriction Enzyme (*Hind*III) Digestion Analysis of HSV-BAC Clones

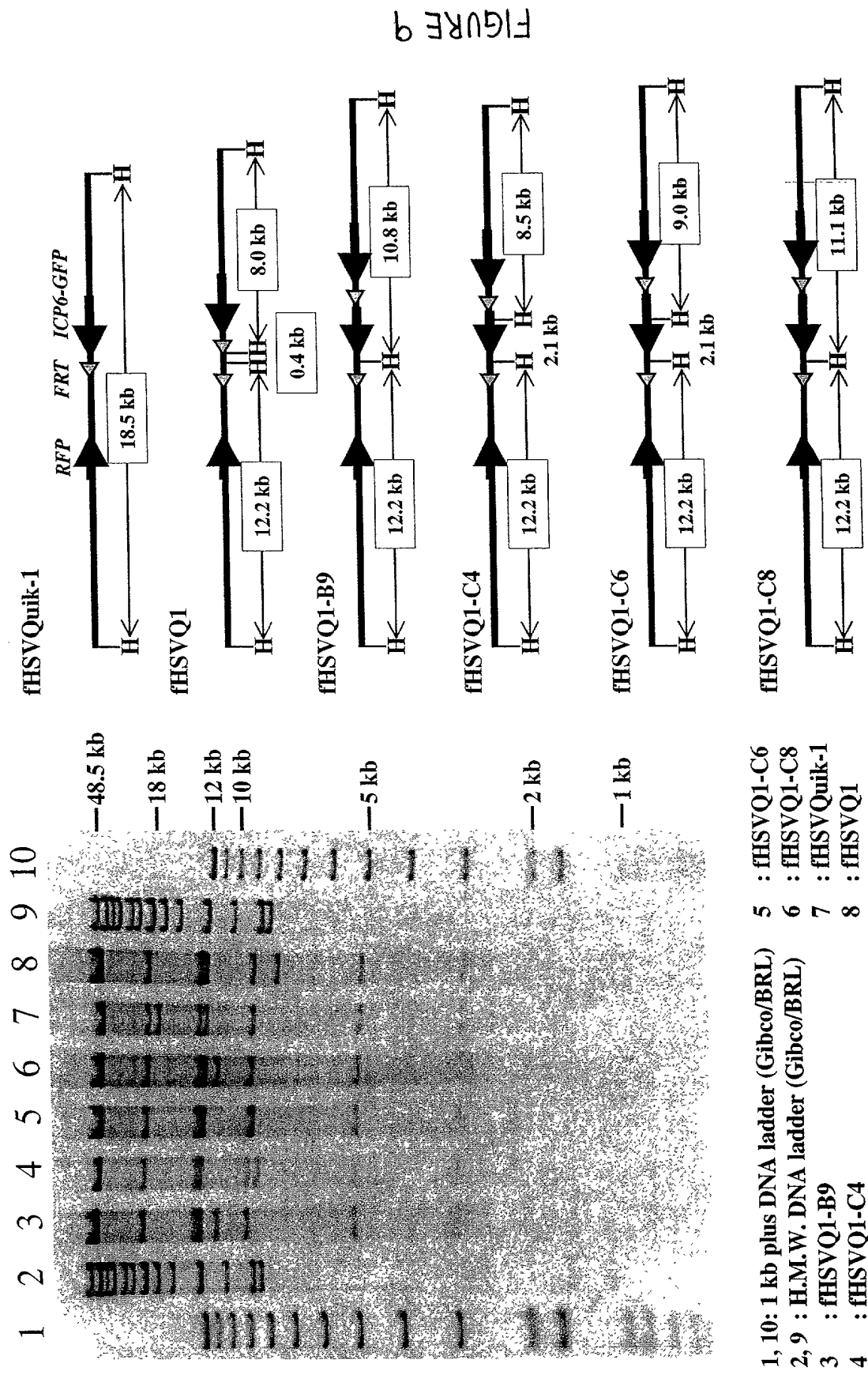
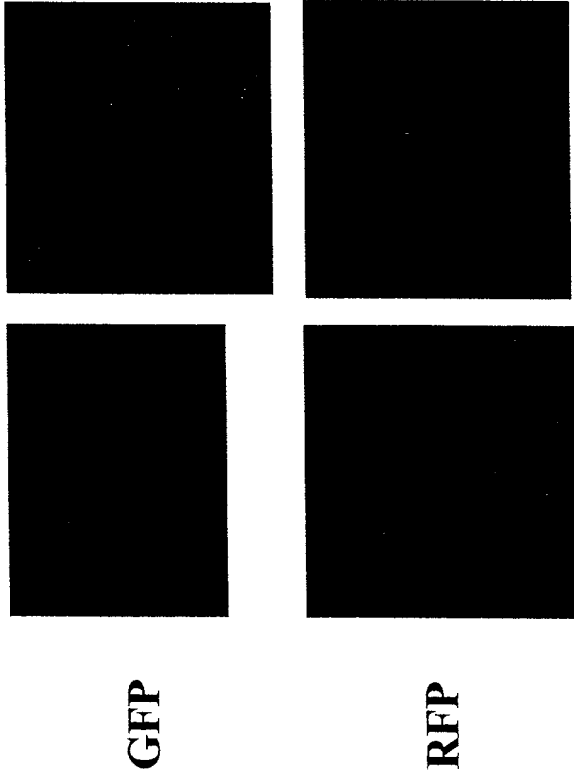


FIGURE 9

# Loss of RFP Expression can be Used as an Indicator for Successful Removal of BAC Backbone

VERO cells were cotransfected with pcnCRE and either fHsvQuik-1 or fHsvQ1. Sixty hours later, the progeny viruses were harvested, serially diluted, and inoculate onto VERO cells plated in 96 well-plates. Viral plaques derived from fHsvQuik-1 showed both GFP and RFP signals, while those from fHsvQ1 showed GFP signal only. This indicates that the prokaryotic backbone of fHsvQ1 flanked by two *loxP* sites was successfully excised.



## Titers of rescued recombinant viruses

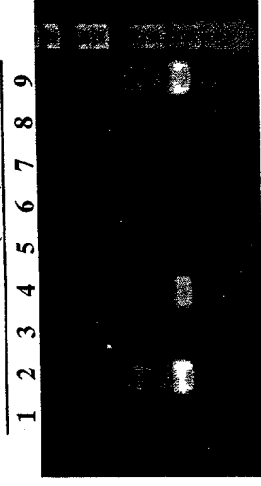
	fHsvQuik-1	fHsvQ1
GFP (+) plaques	1.8 x 10 <sup>5</sup>	2.0 x 10 <sup>5</sup>
RFP (+) plaques	1.8 x 10 <sup>5</sup>	2.5 x 10 <sup>3</sup>

(PFU/mL)

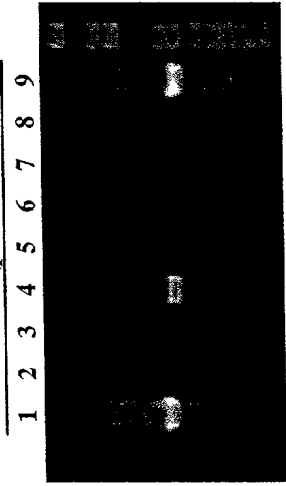
FIGURE 10

# PCR Analysis of Rescued rHsvQ1s

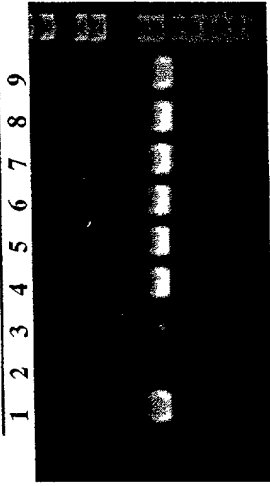
P1/P2 (RFP)



P3/P4 (pTransfer)



P5/P6 (Transgene)



P7/P8 (ICP6-Transgene Junction)

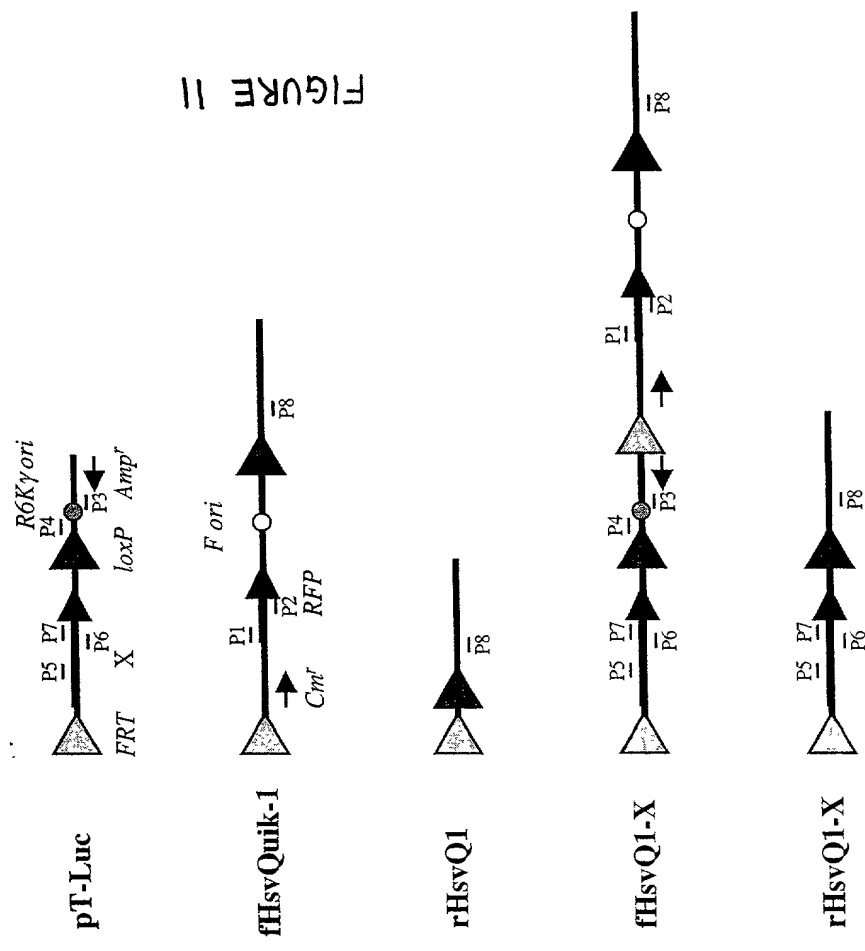
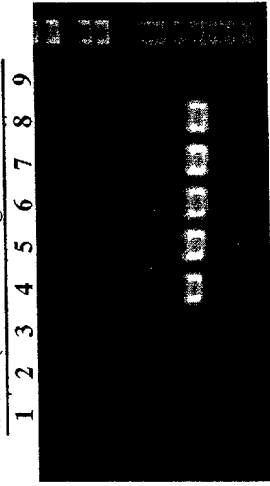
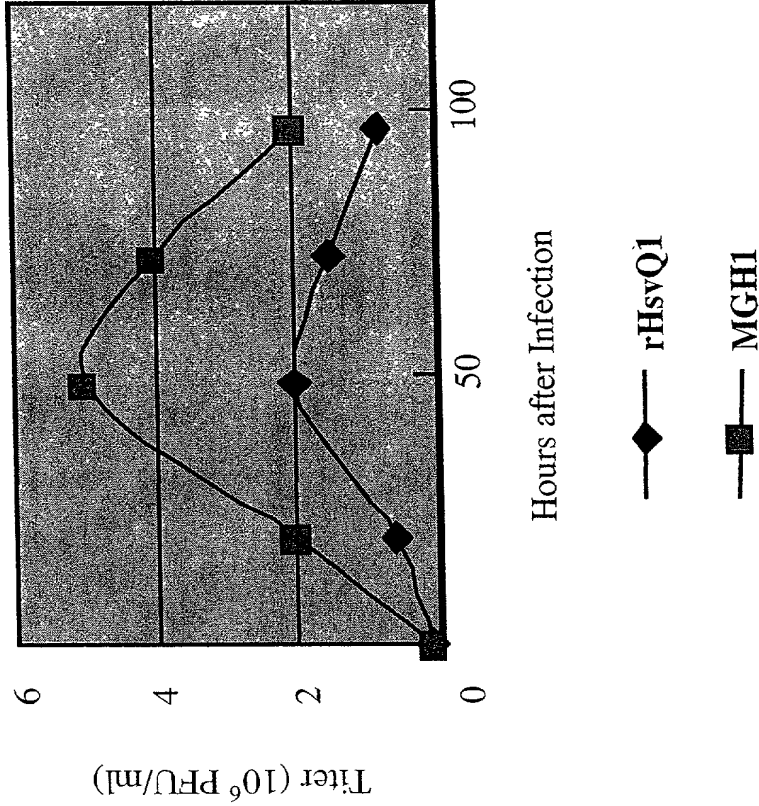


FIGURE 11

- 1: pT-Luc
- 2: fHsvQuik-1
- 3: rHsvQ1
- 4: rHsvQ1-B9 (RFP+)
- 5: rHsvQ1-B9
- 6: rHsvQ1-C4
- 7: rHsvQ1-C6
- 8: rHsvQ1-C8
- 9: fHsvQ1-C8

One-step Growth Curve of rHsvQ1 and MGH1



In vivo safety study on BALB/c mice

Virus: rHsvQ1 v.s. MGH1 (2.5 x 10<sup>8</sup> pfu/ml)  
Animals: 6-week-old female BALB/c mice  
(five animals each group)  
10 µL injected into right striatum  
Duration: 4 weeks  
Results: No animal died in either group  
LD<sub>50</sub> > 2.5 x 10<sup>6</sup> PFU

Cytopathic effect in vitro

	MGH1	rHsvQ1
Human glioma cell lines		
U87ΔEGFR	6.7 %	19.6 %
Gli36Δ5	18.9 %	20.1 %
U343	49.3 %	44.9 %
Normal human fibroblast		
MRC9	82.7 %	90.3 %

(% surviving cells at 48 hours after infection, moi = 0.1)

FIGURE 12

# Restriction Enzyme Digestion Analysis of Mini-Prep DNA

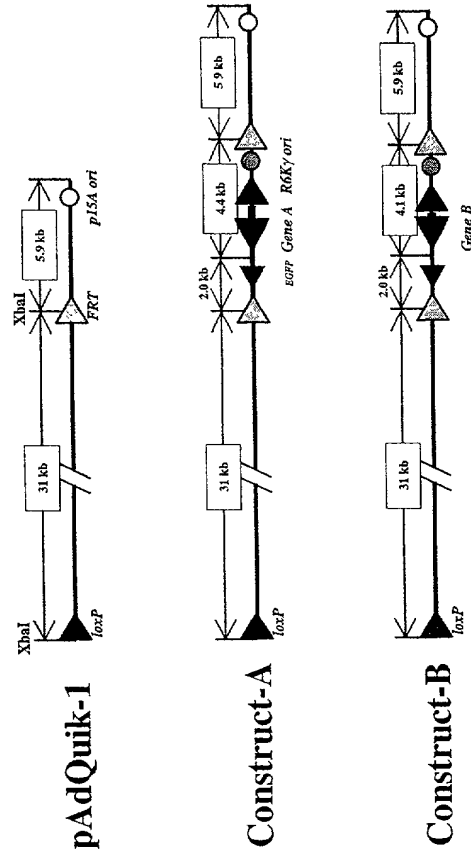
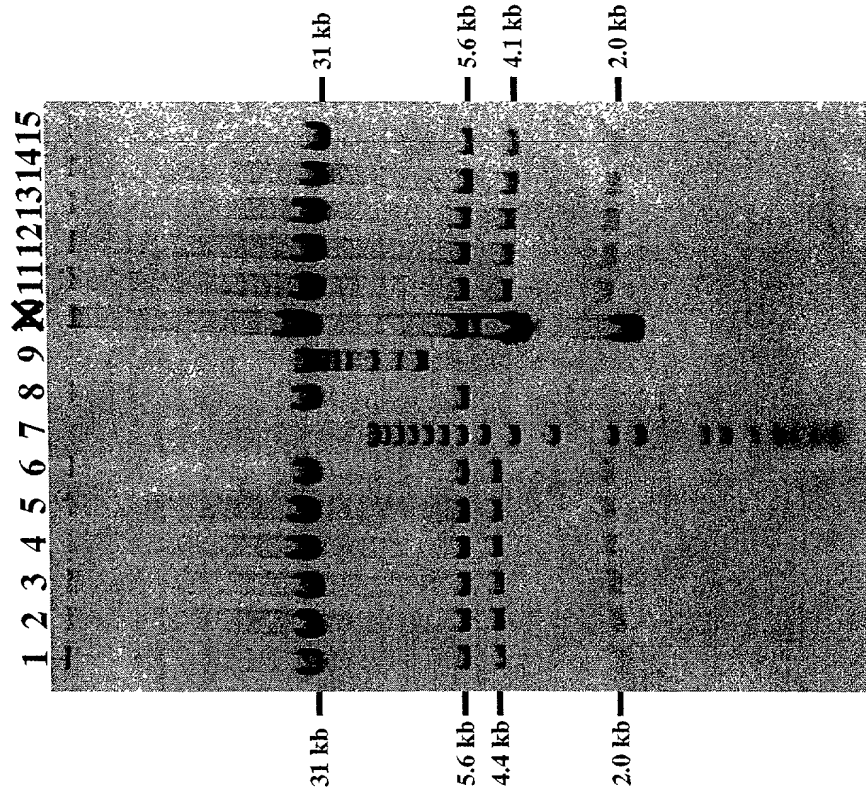


FIGURE 13

Lanes 1-6 : mini-prep (construct-A, XbaI digested)  
 Lane 7 : DNA ladder (Gibco/BRL)  
 Lane 8 : pAdQuik-1 DNA (XbaI digested)  
 Lane 9 : High molecular DNA marker (Gibco/BRL)  
 Lanes 10-15: mini-prep (construct-B, XbaI digested)

# Adenovirus Producing Foci Formation after Transfection

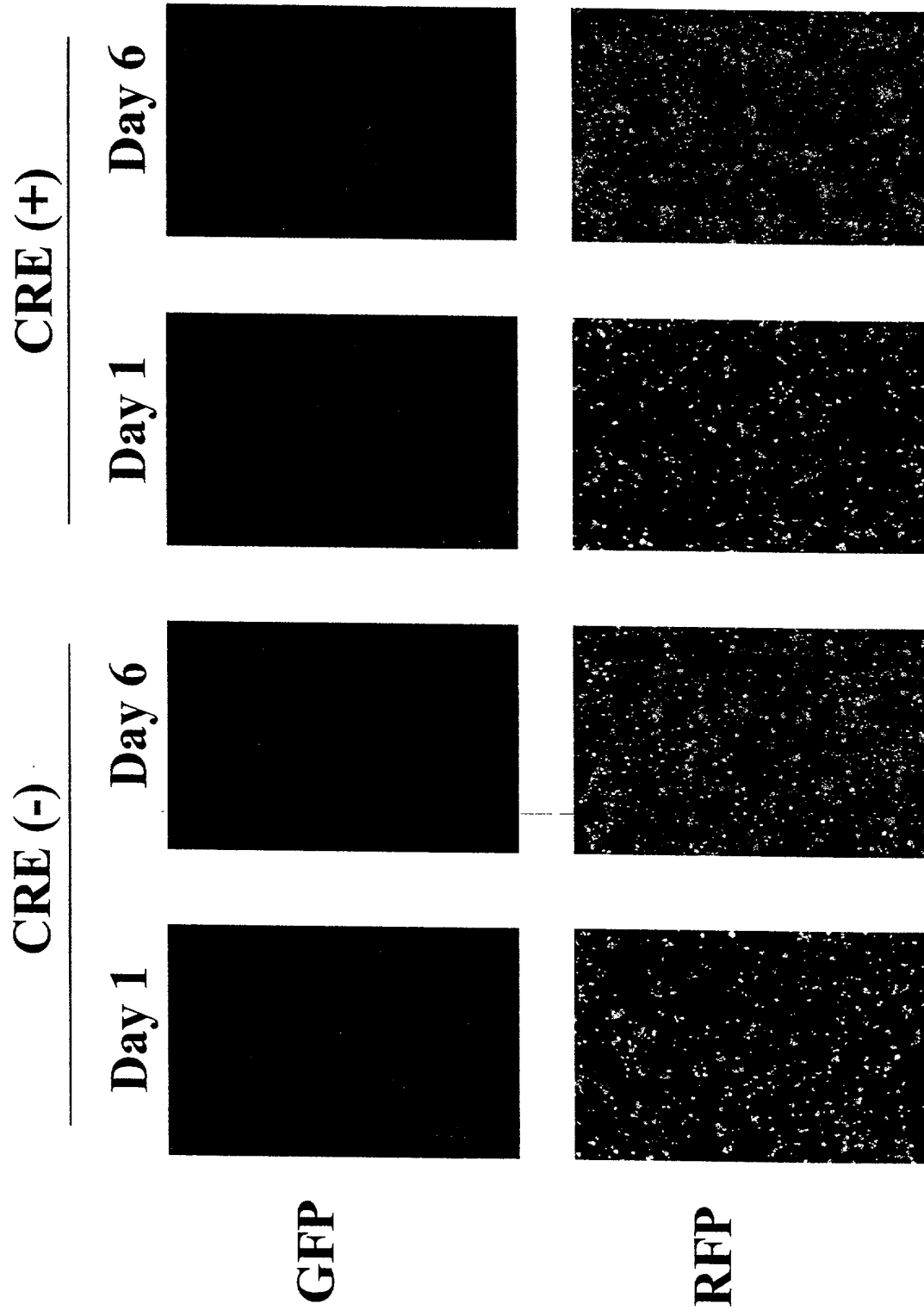
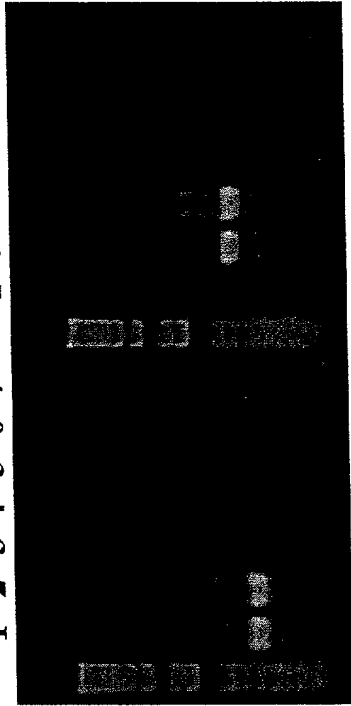


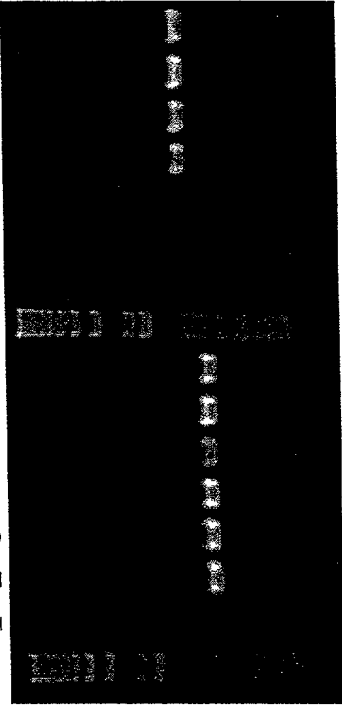
FIGURE 14

# PCR Analysis of Rescued rAdQ1-GC-Luc

P1/P2 ( <i>RFP</i> )							P3/P4 ( <i>pTransfer</i> )						
1	2	3	4	5	6	7	1	2	3	4	5	6	7



P5/P6 ( <i>Luc</i> )							P7/P8 ( <i>Luc-EI</i> )						
1	2	3	4	5	6	7	1	2	3	4	5	6	7

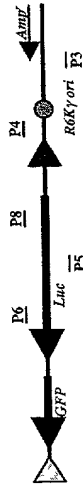


Lane 1 : pAdQuik-1  
Lane 2 : pAdQ1-GC-Luc  
Lane 3 : pT-GC-Luc

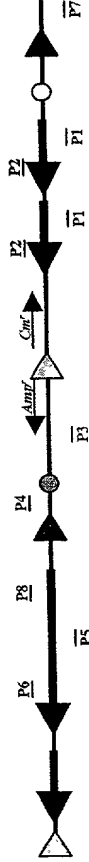
pAdQuik-1



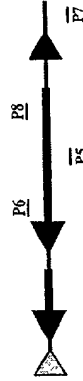
pT-GC-Luc



pAdQ1-GC-Luc



rAdQ1-GC-Luc

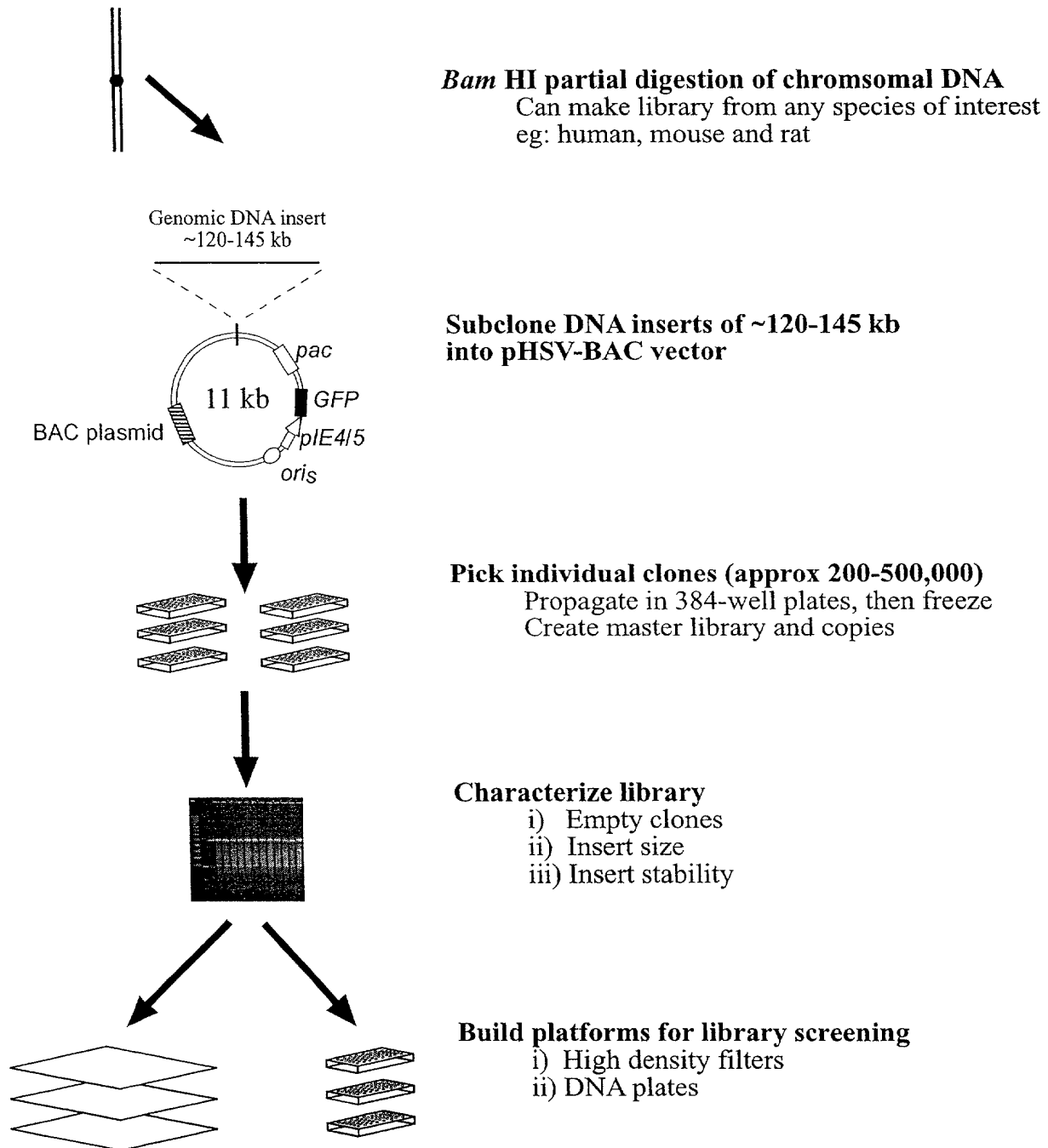


Lane 4 : rAdQ1-GC-Luc clone 1  
Lane 5 : rAdQ1-GC-Luc clone 2  
Lane 6 : rAdQ1-GC-Luc clone 3  
Lane 7 : rAdQ1-GC-Luc clone 4

FIGURE 15

# The pHSV-BAC Library I

## Library construction and characterization



**Figure 16**

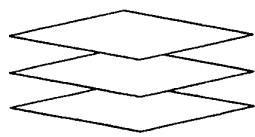


# The pHSV-BAC Library II

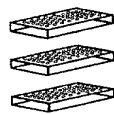
## Screening the library to obtain clones for functional studies

---

204070 "912500T



High density filters

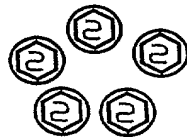
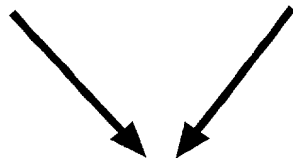


DNA plates

### Screen library to identify clones

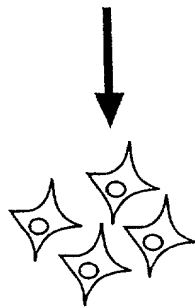
Two screening platforms will be available.

- i) High density filters for hybridization
- ii) DNA plates of pooled DNA for PCR



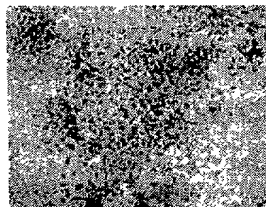
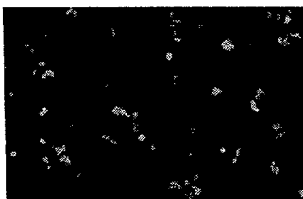
### Package clone as HSV-1 vector

Using improved helper virus-free amplicon packaging system.



### Infect cell line of interest

Human and mouse cell lines or primary cells may be used



### Assay for gene function

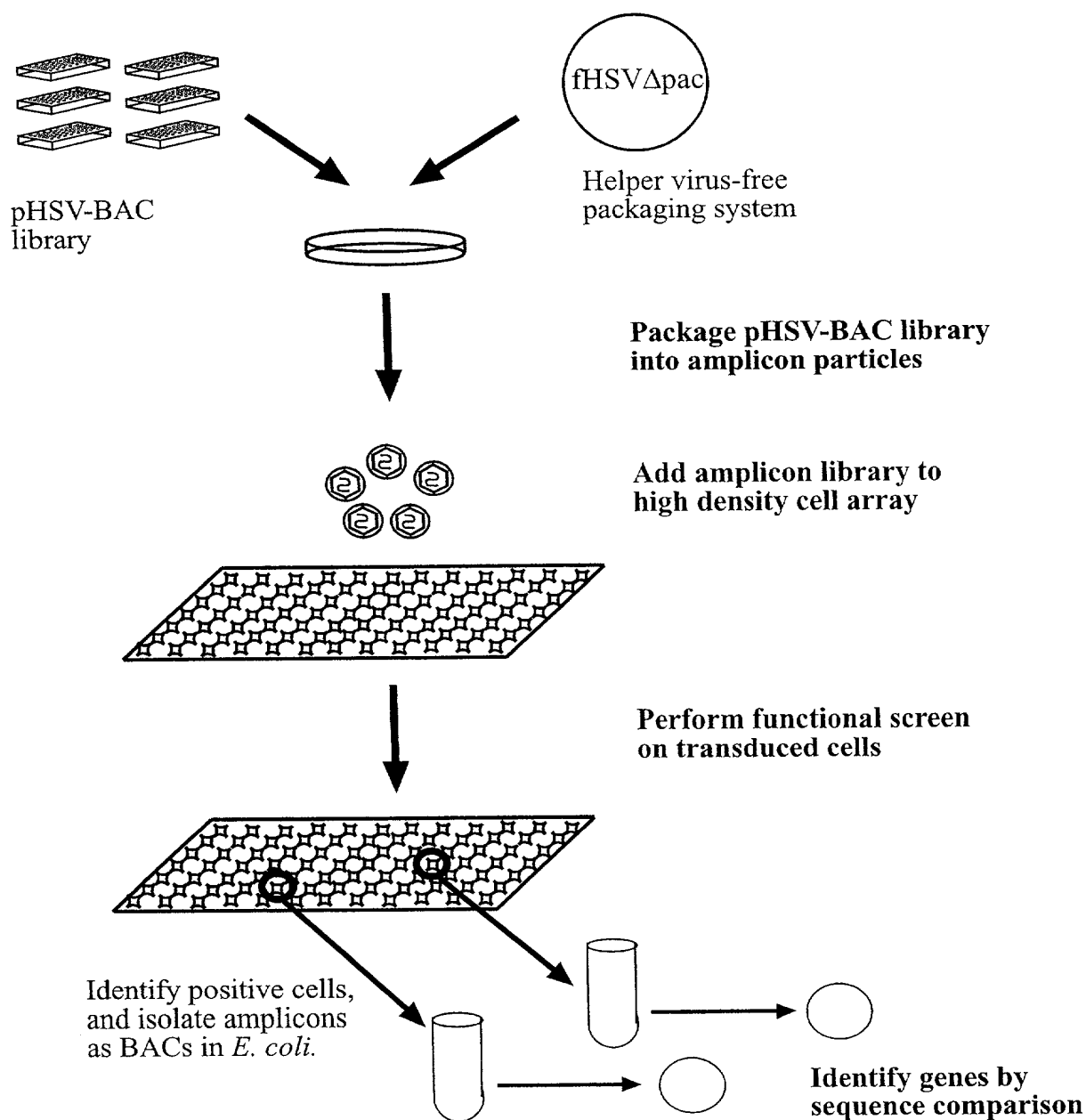
In this example GFP and HPRT expression are shown from the pHSV-HPRT amplicon in a human fibroblast cell line.

**Figure 17**

# The pHSV-BAC Library III

## Library screening by functional assay

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**Figure | 8**